PHOSPHORUS IN BUTTER

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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J. T. Cusick

For a long time manufacturers of butter, and students of its manufacture, have been searching for a method that would give a product capable of withstanding storage conditions and preserving its original quality. The bulk of the methods investigated have dealt with the way in which the raw material is treated and handled before churning. While this earlier treatment does influence the keeping qualities of the churned product, the chemical changes produced, as evidenced in "off flavors," have been investigated in only a few instances. Bacterial, chemical, and enzymic action on butter during its period of storage have been studied almost entirely from the manufacturer's viewpoint, but the results of the methods used by the manufacturer have led to no definite conclusions as to the chemical changes produced by the various manufacturing processes. It is, therefore, with the view of adding some information to present knowledge of the chemical changes occurring in butter during storage, that the rôle played by phosphorus is discussed here. It is clearly shown that the various methods of handling the raw material, and the ways in which it is treated by the manufacturer before churning, have a pronounced influence on the phosphorus in its various forms. One cannot properly interpret "off flavors" and the like without knowing, first, what constituents undergo change, and secondly, what products of decomposition are formed and by what agents.

REVIEW OF LITERATURE

Friis (1897)[2] states that pasteurization of milk or cream appreciably decreases the content of free fatty acids in the resulting butter. His experiments show that

> the amount of free acids in the butter is greatly decreased when the milk or the cream is heated previous to the churning; pasteurization of the milk reduces the free fatty acid content of the fat more than pasteurization of the cream. About half the acidity of fresh sour-cream butter is due to free fatty acids in the butter, and the other half to the acidity of the buttermilk remaining in the butter.

[1] Also presented to the Faculty of the Graduate School of Cornell University, December, 1919, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

The work here described was done in the Department of Agricultural Chemistry of the New York State College of Agriculture at Cornell University, under the direction of Professor George W. Cavanaugh.

[2] Dates in parenthesis refer to *Literature Cited*, page 186.

Schmidt (1898) found that pasteurization of the cream at high temperatures, and salting of the butter, lowered the acidity and the number of bacteria in the butter. Salted butter did not become rancid as soon as did unsalted butter, in his experiments.

Fishy flavor in butter is due to a bacterium, according to O'Callaghan (1902), and is remedied by pasteurization. This theory of the cause of fishy flavor is denied by many investigators, who claim that the flavor is due to enzymic or chemical decomposition.

Steiner (1901) states that pasteurization gives as high a loss of albumin in the butter as 82.5 per cent. The amount of loss depends on the temperature at which the milk is heated and on the time of heating.

Harcourt (1903) found that the amount of nitrogenous matter in butter does not affect its keeping quality.

A primarily practical viewpoint of the question was taken by Lee (1909), who conducted pasteurizing experiments on "farm-skimmed" cream. He concluded that pasteurizing does not affect the body, or texture, of butter, and does not improve the quality of butter made from sour "farm-skimmed" cream.

According to Sayer, Rahn, and Farrand (1908), the use of starter in preparing cream for churning has a decided influence on the butter. Pure cultures of starter gave butter of a high score, according to these investigators. Lindsey (1908) placed great emphasis on care in the quality of the starter to be used, and found that the flavor of butter depends primarily on the cleanliness of the milk, the stage of lactation of the animal, the skill and care of the butter maker, and, especially, the character of the starter employed. He concluded also that normal foodstuffs must be considered of secondary importance in establishing butter flavor.

McKay and Bower (1908) studied the moisture content of butter as it affects the quality of the product. Their efforts led them to conclude that high water content between 15 and 16 per cent does not necessarily mean a low score, nor does less than 13 per cent mean a high score.

Rogers and Gray (1909) made butter from pasteurized and from unpasteurized cream of varying degrees of acidity, and stored it at temperatures of 32°, 10°, and −10° F. They state:

> The butter made from unripened unpasteurized cream always developed a cheesy or rancid flavor. The butter made from ripened cream, both pasteurized and unpasteurized, developed cold-storage, fishy, and other flavors typical of storage butter. In all cases the overripe butter showed marked deterioration. The butter made from pasteurized cream

without starter usually retained its flavor with little or no change. Even at 32° F. the sweet-cream butter deteriorated very little.....

Butter made from pasteurized cream with starter added,..... retained its fresh flavor better than the ripened-cream butter, but was not quite equal in keeping quality to that made from sweet pasteurized cream.

High acidity reduces the keeping quality, according to these workers.

Melick (1909:281) found that "the use of commercial lactic acid as a substitute for starter proved advantageous only when used in very rancid cream....."

Saponification of the butterfat takes place during storage, according to Vincent (1910), increasing the insoluble fatty acids. The soluble and the insoluble fatty acids are formed directly by decomposition and by synthesis, respectively. It is evident that the glycerides of the insoluble acids are changed to a greater degree than are those of the soluble acids. Vincent found also that glycerol is present in old butter and in old cream, but not in milk.

During experiments in the manufacture of butter and cheese, Dean (1910) found that when 10 per cent of starter was added to cream for making butter, there was a greater loss of fat in the pasteurized than in the raw cream. This loss increased with the increase of acidity in the cream at the time of pasteurization. There was little difference in the quality of fresh butter when pasteurized at different temperatures, but the keeping quality was improved by pasteurizing the cream at a temperature between 60° and 82° F.

Larsen, Lund, and Miller (1910) found that the acidity of thoroughly washed butter at the time of making was less than that of butter that was washed but little, by the equivalent of 0.3 cubic centimeter of N/10 alkali to 10 grams of butter. At the end of sixteen weeks this difference had increased to only 0.5 cubic centimeter. Butter made from pasteurized cream did not increase in acidity as rapidly as did butter made from raw cream. The average difference between the two samples immediately after churning was only 0.1 cubic centimeter, but after the butter had been kept in storage for sixteen weeks this difference had increased to 1.1 cubic centimeters. During the sixteen weeks the acidity of the pasteurized-cream butter had increased only 0.6 cubic centimeter, as compared with an increased acidity of 1.6 cubic centimeters in the raw-cream butter. The acidity in lightly salted butter increased by 4.7 cubic centimeters in sixteen weeks, as compared with an increase of 1.6 cubic centimeters

for heavily salted butter. The acidity of ripened-cream butter increased more than did the acidity of sweet-cream butter. The bulletin states (page 715):

> These results indicate that butter from fresh and properly ripened cream not over one day old keeps better than does butter made from sweet cream. The butter fat from very fresh cream is apparently in a more stable condition than is the fat in the sour cream over one day old, and not so predisposed to decomposition. It indicates that butterfat, in the form of butter, keeps better than does butterfat in the form of cream, even though it be in properly ripened cream.

It has been found by Rahn, Brown, and Smith (1909) that there is very little loss of lactose during the storage of butter, and apparently little or no relation between the water-soluble acids and the amount of lactose lost. The butter having the highest initial scores showed the least change in its amino nitrogen content. Butter scoring a little lower and made from cream of a poor quality showed an increased decomposition of protein. The butter with the lowest score yielded the largest amount of protein cleavage products.

The influence of the alkalinity of the wash water was investigated by Meijeringh (1911). He found that the water content of butter washed with alkaline water was higher than that of butter washed with slightly acid water. He states that the butter in the acid solution clumped better than did that in the alkaline solution. The effect of bacteria in the wash water suggested to Melick (1906) the deduction that there is a direct relation between the bacterial content of the wash water used and the keeping quality of the butter.

Kooper-Güstrow (1911) states that butter made from sour cream containing iron rust, has a metallic flavor and a marked grayish color interspersed with dark spots. However, a wash water with an iron content as high as 36 milligrams to 1000 cubic centimeters of water does not affect the quality of the butter.

Rogers and Gray (1909) draw the following conclusions from investigations on pasteurized and on unpasteurized cream of varying amounts of acidity, and on the resulting butter stored at different temperatures:

> Butter frequently undergoes marked changes, even when stored at very low temperatures. These changes are more marked as the acidity of the cream from which the butter is made is increased.
>
> No bacteria were found in the cream or the butter which could reasonably be expected to be the cause of the more rapid deterioration of the high-acid butter.

The changes in the high-acid butter were not checked by heating the ripened cream, which shows that they were not brought about by enzymes secreted with or in the cream and carried into the butter.

Marked changes of an undesirable nature were produced in butter by acidifying pasteurized cream with various acids. These changes did not take place all at once, but were of a progressive nature.

The results indicate that the acid developed normally in the cream by the action of the lactic-acid bacteria, or added directly to the cream in the form of pure acid, brings about or assists in bringing about a slow decomposition of one or more of the labile compounds of which butter is largely composed.

Rogers and his associates (1913) found later that copper sulfate produces fishy flavor in butter. They found also that fishy flavor will develop in cream ripened while in contact with two sheets of copper.

Mortensen, Gaessler, and Cooper (1914) differ with Lee (1909), claiming that pasteurization of either sweet or sour cream improves the flavor of the resulting butter. The protein content of the resulting butter is not influenced by the pasteurization of sweet cream, according to these investigators, but is decreased by the pasteurization of sour cream.

The decomposition of protein in butter during storage has been studied by Brown (1915). He finds that the protein of butter is slowly broken down to amino acids and ammonia. The percentage of total nitrogen in unsalted butter, from the amino acids and ammonia, was found to be 5.71 at first, and 7.59 after the butter had been stored at −6° C. for two hundred and forty days. In the salted butter the amino acids and ammonia increased to 8.19 per cent after a storage period of two hundred and forty days. Hunziker (1916) also found protein hydrolysis in butter during storage, as well as amino acids and other products of decomposition. Hunziker concludes that the initial condition of the cream, the amount of hydrolyzing agents present, and the temperature, determine the degree of hydrolysis.

A new protein, soluble in alcohol, has recently been found in milk by Osborne and Wakeman (1918). It has a phosphorus content, on an ash-free, water-free basis, of 0.08 per cent P. This protein, while soluble in alcohol, is insoluble in dilute acids or in salt solution. The results of the experiments here described seem to show that such a protein exists in butter. In earlier work on phosphatides in milk, Osborne and Wakeman (1915) obtained 116 grams of lecithin from 3000 liters of milk. While they were hydrolyzing a solution of this lecithin with barium sulfate, some of the lecithin was broken down so that trimethylamine was detected in the vapors from the hot solution.

EXPERIMENTAL AND ANALYTICAL RESULTS OF THE PRESENT INVESTIGATION

The butter used in all the experiments here discussed was made from cream containing 30 per cent of butterfat. Seven portions of the cream were churned separately in a barrel churn, operated by hand. The butter, washed and worked in the usual manner, was then divided, one half being packed immediately in porcelain jars, and the other half being salted to a content of $2\frac{1}{2}$ per cent before packing. All samples were then stored in a refrigerator held at −10° C.

Before churning, the seven portions were treated differently, as follows:

Portion 1: To 5 pounds of sweet raw cream there was added 2 ounces of starter — a pure culture of lactic-acid-producing bacteria, in skim-milk. The cream mixture was then held for six hours at room temperature, after which it was placed overnight in a refrigerator at 0° C. The next morning it was removed from the refrigerator, and was brought to a temperature of 15.5° C. by immersing the can in warm water. The mixture had developed an acidity, estimated as lactic acid, of 0.38 per cent. A few drops of butter color were added and the mixture was then churned. The temperature during churning was approximately 15.5° C. After churning, the buttermilk was removed and the butter was washed twice with water at 15.5° C. Each portion of the wash water was equivalent in volume to the buttermilk removed from the churn. In washing the butter, the churn was given only one revolution for each washing. After washing, the butter was removed from the churn with a wooden ladle and was worked for about five minutes in a wooden bowl. It was then divided into two portions, one portion being packed in porcelain jars and the other portion being salted to a content of $2\frac{1}{2}$ per cent. All the samples were labeled, covered with parchment paper, and stored in a refrigerator at −10° C.

Portion 2: The second portion consisted of sweet raw cream which was churned as soon as it was separated from the milk. After churning, the butter in this sample and that in the following samples was treated the same as was the butter from portion 1.

Portion 3: Lactic acid of 25 per cent strength was added to sweet raw cream until the acidity of the cream was 0.38 per cent. The mixture was then heated to 74° C. and held at that temperature for thirty minutes.

The flask containing the cream was then suddenly cooled in water to 15.5° C., and after standing for three hours the cream was churned.

Portion 4: Raw cream was allowed to stand at room temperature until it developed an acidity equivalent to 0.35 per cent, when it was churned. The sample took three days to develop this degree of acidity.

Portion 5: Commercial lactic acid of 25 per cent strength was added to the cream until the acidity of the cream was 0.35 per cent. The portion was thoroughly stirred, quickly brought to a temperature of 15.5° C., and then churned.

Portion 6: Cream was heated in a flask to 74° C. for thirty minutes, was then cooled to 15.5° C., and, after standing for three hours, was churned.

Portion 7: The cream was pasteurized as was portion 6; but instead of being churned after pasteurization, the sample was cooled, was allowed to stand at room temperature for four hours after 4 ounces of liquid starter had been added, and was then placed overnight in a refrigerator at 0° C. After the cream was removed from the refrigerator the next morning, its temperature was raised to 15.5° C. and it was churned immediately. The acidity of the cream at the time of churning was 0.45 per cent.

As may be seen from the above-described methods of treatment of the various portions, the manufacturing methods of treatment of cream before churning were closely followed. The seven methods outlined are the ones most generally employed in the manufacture of butter, and it was with the idea of simulating conditions that would be used in any manufacturing process for butter that these seven portions were treated as described. The different methods employed seem to have varying influences on the phosphorus compounds in the milk and the cream, and also on the churned product during storage.

The phosphorus compounds of milk, which are present also in cream and in butter, are: casein, lecithin, the new protein found by Osborne and Wakeman (1918), and inorganic phosphates. To show the effect on these compounds of the various methods of handling the cream, determinations were made of the organic and the inorganic phosphorus content of the respective butters made from the seven portions of cream.

In an attempt to find a method which would give directly the organic phosphorus in butter, instead of having to obtain it by difference as is the general custom the experiment was made of incorporating the butter

into calcium sulfate and extracting the organic phosphorus compounds with alcohol. The method was as follows: 25 grams of butter was intimately mixed with 150 grams of anhydrous calcium sulfate until all the butter was completely absorbed and the mixture felt dry and powdery to the touch. In some cases a larger quantity of calcium sulfate is necessary to acquire the desired consistency. The mixture was then placed in a large Soxhlet apparatus, a filter paper being used instead of the customary extraction thimble. About 150 cubic centimeters of 95-per-cent alcohol was used as the extracting agent, and the apparatus was run for forty-eight hours. At the end of that time the alcohol was removed, transferred to an Erlenmeyer flask, and evaporated, and the residue,[3] consisting of lecithin, fatty acids, and alcohol-soluble protein, was digested with nitric acid. From this point, method 2 (*b*) in the Methods of Analysis of the Association of Official Agricultural Chemists[4] was used. To the result obtained by the alcoholic extraction is added the per cent of phosphorus obtained from the casein. In making the organic phosphorus determination of the casein, 10 grams of butter was melted and was filtered on a hot funnel, and the filter was washed with three or four portions (30 cubic centimeters each) of 80-per-cent alcohol. The filter paper and its contents were then removed to a 300-cubic-centimeter Erlenmeyer flask with a glass stopper, and were shaken with 75 cubic centimeters of a solution of 0.2-per-cent hydrochloric acid. The supernatant liquid in the flask was then decanted through a dry filter paper, and the extraction of the casein and the filter paper in the Erlenmeyer flask with the acid solution was repeated three times. By this method all the phosphorus compounds of butter, with the exception of the casein, were removed. The new filter was then removed from the funnel and placed in the Erlenmeyer flask with the old filter, and phosphorus determination on the whole was made by the same method as in the case of the alcohol residue. The combined phosphorus results, from the alcohol residue and the casein residue, gave the total organic phosphorus content of the butter.

The low results — 0.0035 and 0.0038 per cent of phosphorus (P_2O_5,) — from the alcoholic residue for total organic phosphorus, seem to indicate that not all the alcohol-soluble phosphorus compounds were extracted. Some,

[3] A test for phosphates must be made on the residue.
[4] Official and provisional methods of analysis, Association of Official Agricultural Chemists, p. 3. U. S. Bur. Chem., Bul. 107. 1908.

if not all, of the lecithin was extracted, and the alcohol residue showed the presence of protein also. However, since the accuracy of the method was not definitely established, its use in obtaining results in this problem was discontinued; it has nevertheless not been discarded entirely, as further work on the method is contemplated. The author has not been able to find any reference to attempts at the direct separation of organic phosphorus from inorganic phosphorus in butter. Attempts have been made at the direct determination of organic phosphorus in milk, and a direct determination of lecithin in milk has been made by Osborne and Wakeman (1915).

The well-known Hart and Andrews method[5] for determination of soluble organic and soluble inorganic phosphorus was applied in a general way to the extraction of the soluble organic phosphorus and the soluble inorganic phosphorus in butter. The method was as follows: 20 grams of butter was melted in a beaker and washed into a separatory funnel with 50 cubic centimeters of a half-saturated sodium chloride solution which had been heated to about 45° C. The separatory funnel was shaken vigorously, the fat was allowed to separate, and the aqueous part was drawn off and passed through a filter paper into a beaker. Two other extractions were similarly made, about 30 cubic centimeters of the salt solution being used in each case. The salt-water extraction was followed by six washings in 25 cubic centimeters of a warm solution of 0.2-per-cent hydrochloric acid. This method extracted all the soluble inorganic phosphorus and an appreciable amount of the organic phosphorus from the butter. The different fractions of the solvents having been filtered together, the filtrate was then divided into two portions. Total soluble phosphorus was determined on one portion according to the method of the Association of Official Agricultural Chemists. Soluble inorganic phosphorus was determined on the other portion according to the Hart and Andrews method. This was done by immediately neutralizing the solution to slight alkalinity with ammonia, precipitating the soluble inorganic phosphorus with magnesia mixture, allowing it to stand in a cool place for four hours, filtering, and ashing. The ash was redissolved in a small amount of hydrochloric acid and hot water, reprecipitated, filtered, ashed, and weighed as mag-

[5] Hart, E. B., and Andrews, W. H. The status of phosphorus in certain food materials and animal by-products, with special reference to the presence of inorganic forms. Amer. chem. journ. **30**: 470–485. 1903.

nesium pyro-phosphate. The soluble organic phosphorus is then the difference between the total soluble and the soluble inorganic phosphorus.

The protein residue from the salt-water and hydrochloric-acid extractions was placed in a 300-cubic-centimeter Erlenmeyer flask and digested with nitric acid. Total phosphoric acid was determined on this according to the method of the Association of Official Agricultural Chemists. This represented the insoluble organic phosphorus in the protein of the butter.

In a few cases the protein residue from the butter, after extraction with hot salt solution and dilute hydrochloric acid, was extracted on the filter with several portions of warm 80-per-cent alcohol, and separate determinations were made on the alcoholic filtrate and on the residue left on the filter. These results are given in table 6 (page 173). They show that some of the protein is soluble in alcohol.

Results of the first series of experiments are given in table 1. These results show the percentages of total soluble, soluble inorganic, and

TABLE 1. PHOSPHORUS CONTENT OF SALTED SAMPLES OF BUTTER MADE ON JANUARY 11 AND ANALYZED ON JANUARY 28, 1918

Butter from cream portion	Per cent of P_2O_5				
	Soluble inorganic	Total soluble	Soluble organic	In protein residue	Total
1	.0129	.0156	.0027	.0123	.0338
2	.0138	.0168	.0030	.0108	.0397
3	.0100	.0124	.0024	.0079	.0249
4	.0127	.0132	.0005	.0131	.0351
5	.0170	.0202	.0032	.0103	.0414
6	.0155	.0180	.0025	.0095	.0344
7	.0108	.0124	.0016	.0089	.0300

soluble organic phosphorus (P_2O_5) in the butter (the last-named obtained by difference), and the percentage of phosphorus in the protein residue. A separate analysis of the total phosphorus content of the portions of butter was made in every case also. The numeral preceding each analysis in the table refers to the portion of cream from which the sample of butter was made.

It is shown by the table that the highest percentage of total phosphorus was retained in the butter when only lactic acid was added and churning

was begun at once (sample 5). There was little difference between this sample of butter and that made from sweet raw cream (sample 2), except that the total soluble phosphorus of sample 5 was greater than that of sample 2. The soluble organic phosphorus content of both was about equal, as were their protein residues also. The only difference in the treatment of the two samples was in the addition of lactic acid to sample 5 before churning. The action of the acid in this case is readily seen in the higher amount of soluble inorganic phosphorus; either there was some splitting-off of phosphorus from some organic phosphorus compound which was heretofore insoluble, or some insoluble phosphates were made soluble by the lactic acid. Sample 4 retained the next highest amount of total phosphorus. This butter was made from raw cream ripened without starter; the acidity developed slowly, at room temperature, at the expense of the lactose. This sample had the greatest amount of phosphorus in the protein residue, however, and the smallest amount of soluble organic phosphorus. The total soluble phosphorus was comparatively very low.

Sample 6, which was made from pasteurized sweet cream, had a total of 0.0344 per cent of phosphorus (P_2O_5). Evidently very little of the organic phosphorus compounds decomposed by heating. The appreciable amount of organic phosphorus seems to be due to the stable condition of the protein and the absence of acid.

Sample 1 was made from raw cream ripened with starter. It had a total phosphorus (P_2O_5) content of 0.0338, and compared very closely with the pasteurized-sweet-cream sample 6, except that its soluble phosphorus was much lower than that of the latter. This difference was made up by the protein residue of sample 1, which was greater in this component by about the same amount as the two samples varied in their soluble phosphorus.

Sample 7 was made from pasteurized cream which was subsequently ripened with starter. It showed a marked depreciation in its total and in its soluble phosphorus. Undoubtedly the heating of the cream during pasteurization rendered many of the phosphorus compounds unstable, so that they were easily broken down by the lactic acid during the long process of ripening before churning. Contrasted with sample 7 is sample 3, which was made from pasteurized sweet cream to which lactic acid had been added. Sample 3 was comparatively low in total phosphorus, due undoubtedly to the high acid content at the time of pasteurization. This proves

that high temperatures, and especially a pasteurizing temperature, accelerate decomposition when acid is present.

The immediate effect of ripening cream with starter showed little decomposition of the phosphorus compounds in the butter, especially in its organic phosphorus content. The sweet raw cream, however, had less splitting-off of phosphorus than had the cream ripened with starter. Raw cream, which was self-ripened, had little soluble organic phosphorus left at the time when it was churned, but later, in storage, the soluble inorganic phosphorus was increased, evidently at the expense of the protein fraction.

The changes that had taken place in the butter samples designated in table 1 after they had been in storage for fifteen months, are shown in table 2. The analyses showed the soluble organic phosphorus to be approaching or to be almost entirely in the inorganic form. The raw

TABLE 2. PHOSPHORUS CONTENT OF SAME SAMPLES IN APRIL, 1919

Butter from cream portion	Per cent of P_2O_5				
	Soluble inorganic	Total soluble	Soluble organic	In protein residue	Total
1	.0158	.0204	.0046	.0120	
2	.0232	.0236	.0004	.0163	
3	.0191	.0193	.0002	.0086	Totals checked
4	.0157	.0168	.0011	.0163	
5	.0227	.0242	.0015	.0166	
6	.0201	.0211	.0010	.0115	
7	.0148	.0207	.0059	.0091	

cream ripened with starter (sample 1) and the pasteurized cream ripened with starter (sample 7) had retained the greatest amounts of organic phosphorus during storage. The phosphorus content of the protein residue in these samples, however, had not increased, and in this respect they showed a marked contrast to the remaining samples, in all of which the phosphorus in the protein residue had increased from a few milligrams in the pasteurized samples 3 and 6 up to 63 milligrams in the sweet-raw-cream sample treated with lactic acid (sample 5). As is generally understood, pasteurization of the cream destroys enzymes and leaves but few

bacteria to survive in the butter and ultimately to produce acid by fermenting the lactose; and since sample 5 had both acid added to the cream from which it was made, and enzymes and bacteria left undisturbed, it may be concluded that the high phosphorus content in its protein residue was due to insoluble phosphorus compounds formed by these agencies. Another factor which may sustain the theory that high acidity produces insoluble phosphorus compounds is evidenced in sample 2 (sweet raw cream), in which a large proportion of the lactose would have been present in the cream before churning and would therefore have been in reserve for the formation of acid in the butter. The presence of salt also seems to have an effect in increasing the insoluble phosphorus compounds during storage.

Analyses of one unsalted sample are given in table 3, to show the effect of the absence of salt on the phosphorus content of butter during storage.

TABLE 3. PHOSPHORUS CONTENT OF UNSALTED SAMPLE OF BUTTER FROM CREAM PORTION 2

Butter sample	Per cent of P_2O_5				
	Soluble inorganic	Total soluble	Soluble organic	In protein residue	Total
Analyzed January 28, 1918.....	.0158	.0199	.0041	.0139	.0468
Analyzed in April, 1919.....	.0229	.0250	.0021	.0147	.....

There was a greater amount of soluble organic and soluble inorganic phosphorus present in this sample, and a larger percentage of phosphorus in the protein residue, than in the salted sample of the same butter. The loss of phosphorus compounds in the salted sample, at this stage of the experiment, was due to the working of the salt into the butter. Later, in storage, there was an increase in the soluble phosphorus, and an appreciable amount of soluble organic phosphorus was present at the end of fifteen months. The protein residue, however, increased its phosphorus content by only a small amount.

Several other samples, duplicates of those designated in table 1 in every respect except that they were not salted, were analyzed and gave results

comparable with those shown in table 3. There were only slight increases in the phosphorus content of the protein residues. This seems to prove that salt is a contributing factor in the production of insoluble phosphorus compounds during the storage of butter. There was in almost every case a larger amount of total phosphorus in the unsalted samples than in the salted ones. As noted above, during the process of salting, the butter loses a small quantity of liquid which carries away with it an appreciable amount of phosphorus compounds.

Another series of experiments, conducted in exactly the same manner as the foregoing, was made about a month later than the first series. The results were almost completely in accord with those of the earlier experiments. These results are given in tables 4 and 5:

TABLE 4. PHOSPHORUS CONTENT OF BUTTER MADE ON JANUARY 31 AND ANALYZED ON FEBRUARY 3, 1918

Butter from cream portion	Per cent of P_2O_5				
	Soluble inorganic	Total soluble	Soluble organic	In protein residue	Total
1	.0125	.0152	.0027	.0133	.0335
2	.0130	.0164	.0034	.0100	.0392
3	.0106	.0138	.0032	.0079	.0318
4	.0102	.0146	.0044	.0122	.0357
5	.0138	.0169	.0031	.0076	.0398
6	.0137	.0167	.0030	.0102	.0386
7	.0078	.0105	.0027	.0069	.0243

TABLE 5. PHOSPHORUS CONTENT OF THREE OF THE SAME SAMPLES IN APRIL, 1919

Butter from cream portion	Per cent of P_2O_5				
	Soluble inorganic	Total soluble	Soluble organic	In protein residue	Total
3	.0191	.0190	.0000	.0092	Totals checked
4	.0199	.0199	.0000	.0157	
7	.0163	.0170	.0007	.0068	

As the protein residue seemed to be affected by the methods of handling the cream and by pasteurization, it was thought advisable to determine whether all the residue was casein, or whether the casein had adsorbed some phosphorus compound, or a portion thereof, which it released only during pasteurization. To determine this point, the samples of butter were successively treated with half-saturated sodium chloride solution and a solution of 0.2-per-cent hydrochloric acid, as in the extraction method previously described (page 167). The protein residue on the filter was removed in the filter paper to an Erlenmeyer flask and shaken with 50 cubic centimeters of 80-per-cent alcohol. The alcohol was then decanted through a dry filter into a clean beaker, and two similar extractions were made on the residue in the Erlenmeyer flask. Finally, the residue and the filter were washed with a small quantity of ether. The filter paper was removed to the Erlenmeyer flask and total phosphorus was determined on this. This was the casein portion. The alcohol-ether filtrate was likewise placed in an Erlenmeyer flask and the total contents were evaporated to dryness. Total phosphorus was then determined on this portion also. The results are given in table 6. Samples 2 and 5 (table 1) were made from cream portions 2 and 5, respectively. They were one month old when these

TABLE 6. PHOSPHORUS CONTENT OF RESIDUE AS SHOWN BY SEPARATION OF ALCOHOL-SOLUBLE PROTEIN FROM CASEIN

Butter from cream portion	Per cent of P_2O_5	
	Protein residue (alcohol-ether extraction)	Casein residue
2 (table 1)	.0030	.0077
5 (table 1)	.0030	.0077
2 (table 4)	.0040	.0075

results were obtained. Sample 2 (table 4) was made from cream portion 2 in the second series, and was three days old when analyzed. All three samples were unsalted.

The results given in table 6 show that an appreciable amount of organic phosphorus can be extracted from the supposed casein residue by alcohol.

This phosphorus compound did not give the tests for lecithin, nor was it soluble in weak salt solution. No further work was done on this alcohol-soluble phosphorus compound except to obtain a positive test for protein. According to the work of Osborne and Wakeman (1918), it appears to be the new protein found in milk by them.

DISTRIBUTION OF PHOSPHORUS AMONG THE VARIOUS PRODUCTS IN CHURNING

A study was made of the distribution of phosphorus among the various products in the manufacture of butter. Results obtained from churnings in which the cream was not pasteurized and was ripened with starter, are shown in tables 7, 8, and 9.

The history of the sample used for the data given in table 7 was as follows: To 20 pounds of sweet cream from milk recently skimmed, a

TABLE 7. DISTRIBUTION OF PHOSPHORUS CONTENT IN BUTTER MADE ON FEBRUARY 22, 1919. ANALYSES STARTED ON THE SAME DATE

	Per cent of P_2O_5			
	Total soluble	Soluble inorganic	Soluble organic	Total
Milk	.1387	.1178	.0209	.2161
Cream	.1033	.0902	.0131	.1619
Buttermilk	.1222	.1143	.0079	.2155
First wash water	.0037	.0031	.0006	.0115
Second wash water	.0000	.0000	.0000	.0004
Salt water*	.0263	.0251	.0012	.0340
Butter	.0191	.0154	.0037	.0485
Washed butter	.....	.....	.....	.0089
Extracted butter	.....	.....	.....	.0038

*Only 100 cubic centimeters of salt water was expressed from the butter.

pint of liquid starter was added. The mixture was allowed to stand at room temperature for three hours and was then placed in a refrigerator overnight. The next morning it was removed from the refrigerator and was found to have developed an acidity of 0.48 per cent. It was then churned in the usual manner, was salted, and was stored at a temperature of 0° C. The fat content of the cream at the time of churning was 24.5 per cent. After five months in storage the sample scored 91, which is considered a high score for storage butter.

There was 49.5 per cent of fat in the cream from which the butter used for the data given in table 8 was made. The cream was fairly fresh and was ripened with starter. The acidity of the cream at the time of

TABLE 8. DISTRIBUTION OF PHOSPHORUS CONTENT IN BUTTER MADE ON APRIL 5, 1919. ANALYSES STARTED ON THE SAME DATE

	Per cent of P_2O_5			
	Total soluble	Soluble inorganic	Soluble organic	Total
Cream	.0689	.0599	.0090	.0905
Buttermilk	.1025	.0960	.0065	.1657
First wash water	.0076	.0058	.0018	.0170
Second wash water	.0038	.0028	.0010	.0050
Salt water*	.0163	.0153	.0010	.....
Butter	.0280	.0205	.0075	.0325

*Only 10 cubic centimeters of salt water was expressed from the butter.

churning was 0.52 per cent. The salt content of the butter was 3.8 per cent. Only 10 cubic centimeters of salt water was expressed from this butter in working it. The analysis of the first wash water from this churning is given in table 9:

TABLE 9. ANALYSIS OF FIRST WASH WATER FROM BUTTER CHURNING
(From butter used for data given in table 8)

Substance	Per cent
Lactose	0.45
Total solids	0.602
Ash	0.050
Lactoglobulin	Present
Lactalbumin	None

Experiments were made on several portions of the butter used for the data in table 7, to determine the effect of varying amounts of salt on the keeping qualities of the product. The portions were salted so as to give the following salt content: 0.5 per cent, 1 per cent, 2 per cent, 3 per cent, 5 per cent, 7 per cent, 10 per cent, and 15 per cent. A fresh (unsalted) sample also was included in the series. The samples were stored in porce-

lain jars in a refrigerator held at 0° C., and were scored every two weeks for quality. The effect of the various salt concentrations on the soluble phosphorus compounds during the storage period of the butter is shown in table 10:

TABLE 10. INFLUENCE OF SALT CONCENTRATION ON SOLUBILITY OF THE PHOSPHORUS COMPOUNDS IN BUTTER

(Butter made on February 22, analyzed on May 25, 1919)

Salt concentration	Per cent of P_2O_5		
	Total soluble	Soluble organic	Soluble inorganic
Unsalted	.0200	.0041	.0159
2 per cent	.0280	.0031	.0249
5 per cent	.0274	.0018	.0256
10 per cent	.0255	.0000	.0255
15 per cent	.0242	.0000	.0239

The samples of washed and extracted butter shown in table 7 were treated by eliminating from them as far as possible all the salt-soluble compounds, in order to show what would be the effect on the keeping qualities. For the washed butter, 100 grams of the butter was treated on the work board with small amounts of a saturated salt solution until about 300 cubic centimeters of the solution had been used. The salt solution was thoroughly mixed with the butter by means of a wooden ladle, and then drained off. The fat was not melted nor removed from the board at any time until it was finally put into the storage jar. It will be seen that in this sample of butter the phosphorus content was reduced, by washing, from 0.0485 per cent (P_2O_5) to 0.0089 per cent. However, it must be admitted that insoluble matter was carried away in the salt solution by this method.

In the case of the extracted butter, the sample was first treated in the same manner as was the washed butter. It was then melted in an evaporating dish, and the protein and the fat were separated by means of a hot saturated salt solution in a separatory funnel. The salt-water-insoluble residue was caught on a hard filter paper, and the salt water from the repeated salt-water extractions of the fat was used to wash the filter. Finally, the protein residue was scraped from the filter paper and mixed

in a jar with the extracted fat. Enough saturated salt solution was then added to equal the water content of the original butter, and the mixture was stirred, hardened, and stored.

Both these samples, the washed and the extracted, were prepared to demonstrate the possibilities that may occur when butter is overworked, and also to show the effect of the absence of salt-water-soluble compounds on the keeping qualities of butter. The only comment made on either of these samples by the scorers, was that the butter was "tallowy." This was of course due to handling.

No fishy flavor was developed in the samples of butter used for tables 7 and 10, despite the fact that salt is a contributing factor in the development of fishy flavor in butter during storage. As lecithin is soluble in salt solution, the reason for washing and extracting the butter samples was to remove this phosphorus compound and note whether fishy flavor developed in the samples treated in this manner. The samples included in table 10 were used to ascertain whether butter samples of varying salt content would develop fishy flavor more quickly in one salt concentration than in another. These samples were used also to test the influence of the varying salt concentrations on the soluble phosphorus compounds in the butter. No flavor which in any way resembled fishy flavor was detected in any of the samples, but table 10 shows the influence of the various salt concentrations on the soluble phosphorus compounds.

It is the author's opinion, however, that fishy flavor in butter is caused by the decomposition or partial decomposition of the lecithin before churning, and that through the solvent agency of the salt solution the products of decomposition are further broken down in storage to trimethylamine. Trimethylamine may in turn be broken down, or it may be completely adsorbed by some other compound. This latter theory is advanced from the fact that fishy flavor is transitory in some butter.

As evidence of the breaking-down of lecithin in butter during storage, it is only necessary to compare the soluble organic phosphorus content of the butter in table 1 with the soluble organic phosphorus content of the same butter analyzed fifteen months later as shown in table 2. The long period of storage reduced the soluble organic phosphorus almost entirely to the inorganic form in all the samples except 1 and 7, which had a pure lactic acid culture added. Samples 1 and 7 held their soluble organic phosphorus during storage, and there was no decomposition of the organic

phosphorus which became soluble during storage. The lactic-acid bacteria seem to protect the soluble organic phosphorus from decomposition.

Lecithin is soluble in sodium chloride solution, and it is claimed that fishy flavor in butter is caused by the breaking-down of this compound while in solution with salt. The handling of the milk and cream before churning influences the solubility of the lecithin, and its decomposition, both before and after churning, is clearly shown by the records in tables 1 and 2. Its decomposition in butter during storage necessarily follows, as the records show also. Lewkowitsch (1914:802) says, in referring to the lecithin content of butter: "Lecithin has been stated by various observers to occur in butter fat to the extent of 0.017 or even 0.15 to 0.17 per cent (calculated from phosphoric acid). Wrampelmeyer stated 0.007 to 0.033 per cent of lecithin. Jaeckle, however, showed that butter fat contains no compound of phosphorus." These results seem to supply additional proof that lecithin is a variable compound in butter, and give emphasis to the author's conclusions that the methods of handling the milk and cream before churning determine the quantity of lecithin in the resulting butter. Also, the rate at which the lecithin is decomposed during storage is determined by the condition and the handling of the milk and cream before churning. However, the rôle played by *Bacterium lactis acidi* in protecting the soluble organic phosphorus from decomposition is not clearly understood. That some organisms have a specific action on phosphorus compounds has been observed, and a summary of the investigation of this subject is contained herein.

Butter from cream portion	Per cent of P_2O_5				
	NaCl extract, inorganic	NaCl extract, soluble	HCl extract of protein	Protein residue after extraction	Butter-fat residue
1	.0107	.0153	.0051	.0120	.0022
2	.0179	.0183	.0053	.0163	None
3	.0145	.0147	.0046	.0086	Trace
4	.0096	.0107	.0061	.0163	Trace
5	.0189	.0204	.0038	.0089	Trace
6	.0148	.0158	.0053	.0115	Trace
7	.0087	.0148	.0059	.0091	.0030

Since it may be of interest to some readers to know what amounts of phosphorus were extracted separately by the various extracting agents, table 2 is given in detail on the opposite page.

BACTERIOLOGICAL INVESTIGATION AS TO THE CAUSE OF FISHY FLAVOR IN BUTTER

Since butter contains lecithin — a mononitrogenous monophosphatid — which on decomposition yields glycerophosphoric acid and choline,— the latter further decomposing to methylamine, ammonia, carbon dioxide, and methane,— it can naturally be inferred that the decomposition products of choline are the cause of the fishy flavor found in some butter. Trimethylamine, which tastes and smells like decomposed fish and which is a decomposition product of choline, is believed to cause fishy flavor in butter. And, since both fishy odor and fishy flavor have been produced in butter when the cream from which the butter was made was inoculated, it can only be assumed that the phosphorus compound which decomposes to trimethylamine is broken down or used as pabulum by the bacteria. The bacterium used in the author's experiment was the one isolated by Hammer (1917) from a can of fishy-flavored milk and named *Bacterium ichthyosmius.*

PLAN OF THE EXPERIMENT

Cream containing 30 per cent of butterfat was treated in the following ways:

Portion D: 750 cubic centimeters of sweet cream was pasteurized at 63° C. for thirty minutes, cooled to 9° C., inoculated with 1 cubic centimeter of an aqueous solution of *Bacterium ichthyosmius,* and churned at once.

After churning, the butter in this case and in all the following treatments was divided, one-half being salted and the other half left unsalted. A check sample (uninoculated and salted) was made also, and all three samples were stored at −10° C.

Portion E: Sweet raw cream was inoculated in the same way as was portion D, incubated at 37° C. for three hours, cooled to 15° C., and churned.

Portion K: Sweet cream was inoculated, and was then incubated at 37° C. for three hours. At the end of that period the acidity was neutralized to phenolpthalein with sodium hydroxide, and the sample was cooled to 15.5° C. and then churned.

Portion N: Cream was inoculated, incubated, and neutralized as was portion K. After neutralizing, one ounce of liquid starter was added, and the mixture was allowed to stand at 15.5° C. for three hours and then churned.

Portion R: Cream was inoculated, one ounce of starter was added, and the cream was allowed to stand at room temperature for three hours to ripen. It was then neutralized and churned.

Portion X: Cream was inoculated, incubated at 37° C. for three hours, and cooled to 15.5 C., and one ounce of starter was added. After three hours the cream was churned.

Each portion of cream was thus represented by an unsalted sample and a salted sample of butter inoculated with *Bact. ichthyosmius*, and a check sample (salted) which was not inoculated. These were all stored at −10° C. Every two weeks during a period of eight months, a count was taken of the bacteria in both the inoculated samples from each portion. All three samples from each portion were scored frequently for quality (flavor).

RESULTS

None of the check samples (uninoculated) were scored as fishy. None of the unsalted samples (inoculated) were scored as fishy. The salted sample from portion D (inoculated), made from fresh cream which was pasteurized and then churned at once, was not scored as fishy by any of the four judges. The bacteria count decreased rapidly both in the salted and in the unsalted sample of portion D.

Samples from portion E were not scored as fishy by the judges. The bacteria count on the salted inoculated sample was 120,000,000 at the time when it was churned, and at the end of six months the count was 460,000. The unsalted sample had a count of only a few bacteria at the end of four months.

The score for portion K was as follows:

Judge	Unsalted sample	Salted sample
No. 1	No comment	Metallic
No. 2	No comment	No comment
No. 3	No comment	Fishy
No. 4	No comment	Fishy

The only difference between the treatment of the cream in this portion and that in portion E was in the neutralization of the cream after incubation.

In portion N there was no comment on the quality of the sample of unsalted butter. The salted inoculated sample was accidentally destroyed at the beginning of the fourth month in storage. The writer, however, had scored the sample as fishy a short time before it was destroyed.

Portion R scored as follows:

Judge	Unsalted sample	Salted sample
No. 1	No comment	Fishy
No. 2	No comment	Oily
No. 3	No comment	Fishy
No. 4	No comment	Fishy

After four months in storage the salted inoculated sample of this portion had a strong fishy odor. At the end of seven months this odor had to some extent disappeared.

The samples from portion X scored as follows:

Judge	Unsalted sample	Salted sample
No. 1	No comment	Metallic
No. 2	No comment	Metallic
No. 3	No comment	Tallowy
No. 4	No comment	Fishy

The bacteria in the salted sample of portion X numbered several millions even after the sample had been in storage for some months.

In the samples of butter just described, fishy flavor was produced by inoculating the cream with *Bacterium ichthoysmius*. The handling of the cream before churning affected the growth of the bacteria differently in every portion. The sweet-cream butter from portion D decreased in its bacteria count very rapidly, and retained a good flavor after being stored for eight months. Portion D compares fairly closely with portion 6 of the earlier experiments as to the way in which it was treated before

churning. The sample from portion 6 retained a large proportion of its total phosphorus, and the soluble organic phosphorus suffered little decomposition, if any. The butter from portion E may be compared with that from portion 2 of the earlier experiments. The total phosphorus content in sample 2 was very high and the amount of soluble organic phosphorus was large. The samples that were scored as fishy in these experiments (from portions K, R, and X) underwent treatment similar to that of sample 4 in table 1. Sample 4 showed a very small amount of organic phosphorus in the soluble form, and it may be inferred that some of its soluble organic phosphorus had been decomposed. All three samples (K, R, and X) prove that where there is a loss of soluble organic phosphorus, fishy flavor develops in the butter. Sample 4 in table 1 also developed a fishy flavor in storage.

Since it seemed necessary to identify the trimethylamine directly, a sample of butter which had been scored as fishy was selected and the trimethylamine was extracted and identified. The method used was as follows: 100 grams of fishy butter was extracted in a separatory funnel with several portions (about 50 cubic centimeters each) of hot water. The washings were separated from the fat and placed in a liter flask. About 25 cubic centimeters of 1:1 caustic potash solution was added to the contents of the flask, the flask was attached to a condenser, and the solution was heated. The distillate was caught in about 50 cubic centimeters of N/10 sulfuric acid solution. The distillation was continued until the volume of the distillate measured about 200 cubic centimeters. The distillate was then evaporated, on a water bath, to dryness. The residue was taken up with a small quantity of 95-per-cent alcohol and again evaporated to dryness. Dehydrated alcohol was then added to the residue, and the extractions were passed through a filter paper into a clean evaporating dish. Several extractions with alcohol were made, after which the new filtrate was evaporated to dryness on a water bath in a covered hood. The new residue, consisting of trimethylamine sulfate, was dissolved in a small quantity of warm water and the contents of the evaporating dish were washed into a 300-cubic-centimeter Erlenmeyer flask. About 5 cubic centimeters of 1:1 caustic potash solution was added to the contents of the flask, and the whole was distilled into 50 cubic centimeters of N/50 hydrochloric acid. A few drops of the distillate were carefully mixed on a glass slide with a solution of platinic chloride,

and orange-colored crystals, octahedral in shape, were produced. Blank checks on the reagents and the water used showed no evidence of contamination with ammonium or potassium, both of which yield crystals of this type. To check the results, a solution of trimethylamine hydrochloride was prepared for comparison, and this gave results identical to those of the trimethylamine salt obtained from the fishy-flavored butter.

As the experimental data show, when cream is handled in certain ways the organic phosphorus compounds which are soluble in salt solution are decomposed more or less before churning or soon after the resulting butter is placed in storage. The organic phosphorus compound lecithin, which is soluble in sodium chloride solution, represents almost entirely, if not entirely, the soluble phosphorus in the organic form as shown in table 1. This soluble organic phosphorus gradually suffers decomposition, as may be seen in table 2. With the exception of two cases, samples 1 and 7, all the samples that had a significant amount of soluble organic phosphorus suffered decomposition of this component during storage; and in the experiment with *Bacterium ichthyosmius*, the butter which, from the method of handling the cream before churning, could be compared to samples 1 and 7, did not develop the fishy flavor. The butter that did decrease in its soluble organic phosphorus content as shown in tables 1 and 2, may be compared to those samples that became fishy in the inoculation experiment.

This publication deals only with the phosphorus in butter, in its various compounds. However, it is an appreciated fact that there are compounds of other salts, and of calcium especially, which have a marked influence on the keeping qualities of butter. Lactic acid also must play no small part in bringing about chemical changes in butter. Further studies are being made on the keeping qualities of butter as affected by calcium compounds and lactic acid.

SUMMARY

For the experiments described in the foregoing pages, butter was made from cream treated in the following ways: sweet cream ripened with starter; sweet cream churned without starter; lactic acid added to cream and the mixture pasteurized; raw cream self-ripened; lactic acid added to cream and churning begun at once; sweet cream pasteurized and then churned immediately; pasteurized sweet cream ripened with starter.

All the methods of handling had some influence on the phosphorus compounds in the cream and subsequently on the phosphorus compounds in the stored butter. Pasteurization had the most decided influence. When cream containing acid was pasteurized, an appreciable amount of phosphorus was rendered soluble and lost in the buttermilk and the wash waters. Much of this phosphorus evidently came from the insoluble protein residue. Pasteurized sweet cream suffered but little phosphorus loss except in the protein residue. More phosphorus was lost when pasteurized sweet cream was subsequently ripened with starter. In the unpasteurized samples, sweet-raw-cream butter retained the largest amount of phosphorus, butter made from raw cream ripened without starter was next, while butter made with starter ranked third.

After fifteen months the samples were again analyzed, and the analyses showed that the phosphorus in the organic compounds had broken down to the inorganic form. Exceptions to this were in the butter made from raw cream ripened with starter, and in the butter made from pasteurized sweet cream subsequently ripened with starter. With the exception of these two, all the samples increased in the phosphorus content of the protein residue. All samples, without exception, increased in soluble inorganic phosphorus during storage. The organic phosphorus compounds in the unsalted samples were slower to break down than were the organic phosphorus compounds in the corresponding salted samples.

There seems to be plenty of evidence that an alcohol-soluble protein containing phosphorus exists in butter and is closely related to casein.

From the results obtained with the samples of butter containing varying amounts of sodium chloride, it can be inferred that salt has an accelerating action on the solubility of insoluble organic phosphorus compounds.

About two-thirds of the total phosphorus of the cream is retained in the buttermilk, and the remaining one-third is shared by the wash waters, the salt exudates, and the butter. The butter finally retains about one-quarter of the phosphorus originally present in the cream.

After fifteen months in storage, all the phosphorus compounds in the fat could be extracted by shaking in a separatory funnel with half-saturated sodium chloride solution.[6] A 0.2-per-cent solution of hydrochloric acid was found necessary to extract the soluble phosphorus in the protein residue.

[6] Extraction with the aid of centrifugation was not tried on the samples used for table 1.

The substance that produces fishy flavor in butter, is undoubtedly preformed in the cream by the breaking-down of the lecithin. It may be assumed that through the solvent action of salt water and lactic acid, trimethylamine (the constituent giving fishy flavor) is formed from one of these broken-down fractions.

CONCLUSIONS

1. In churning, about one-fourth of the total phosphorus of the cream is retained in the butter made therefrom. The remaining three-fourths is lost in the buttermilk, wash waters, and exudates during the salting process.

2. The methods of treatment of milk and cream before churning have an influence on the amount and the form of phosphorus retained in the butter.

3. In storage the soluble organic phosphorus compounds break down, giving inorganic phosphorus compounds.

4. The methods of treatment of milk and cream before churning determine how soon after storage organic phosphorus compounds will assume the inorganic form.

5. Salt in butter has a marked effect in bringing about protein decomposition during storage, even at a temperature of −10° C.

6. The new protein of milk which is soluble in alcohol exists also in butter.

7. Under certain conditions, bacteria are the controlling factors in bringing about chemical changes in the phosphorus compounds of butter.

8. The breaking-down of lecithin and the forming of trimethylamine is the cause of fishy flavor in butter.

9. When fishy flavor develops in butter there is always an appreciable loss of soluble organic phosphorus.

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